Enterovirus infection during pregnancy is inversely associated with atopic disease in the offspring

Korhonen, Laura

2018-12

Korhonen , L , Seiskari , T , Lehtonen , J , Puustinen , L , Surcel , H-M , Haapala , A-M , Niemelä , O , Virtanen , S M , Honkanen , H , Karjalainen , M , Ilonen , J , Veijola , R , Knip , M , Lönnrot , M & Hyöty , H 2018 , ' Enterovirus infection during pregnancy is inversely associated with atopic disease in the offspring ' , Clinical and Experimental Allergy , vol. 48 , no. 12 , pp. 1698-1704 . https://doi.org/10.1111/cea.13280

http://hdl.handle.net/10138/307712
https://doi.org/10.1111/cea.13280

publishedVersion

Received: 24 January 2018 | Revised: 12 August 2018 | Accepted: 1 September 2018

DOI: 10.1111/cea.13280

ORIGINAL ARTICLE

Epidemiology of Allergic Disease

WILEY

Enterovirus infection during pregnancy is inversely associated with atopic disease in the offspring

Laura Korhonen[1,2,3] | Tapio Seiskari[4] | Jussi Lehtonen[1] | Leena Puustinen[1] | Heljä-Marja Surcel[5,6] | Anna-Maija Haapala[4] | Onni Niemelä[7] | Suvi M. Virtanen[8,9,10,11] | Hanna Honkanen[1] | Mira Karjalainen[12] | Jorma Ilonen[13,14] | Riitta Veijola[12] | Mikael Knip[15,16,17,18] | Maria Lönnrot[1,2,3] | Heikki Hyöty[1,4]

[1]Department of Virology, Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland

[2]Department of Dermatology, Tampere University Hospital, Tampere, Finland

[3]Allergy Centre, Tampere University Hospital, Tampere, Finland

[4]Department of Clinical Microbiology, Fimlab Laboratories Ltd, Tampere, Finland

[5]National Institute for Health and Welfare, Oulu, Finland

[6]Biobank Borealis of Northern Finland, Oulu University Hospital, Oulu, Finland

[7]Department of Laboratory Medicine and Medical Research Unit, Seinäjoki Central Hospital and University of Tampere, Seinäjoki, Finland

[8]Unit of Nutrition, National Institute for Health and Welfare, Helsinki, Finland

[9]Faculty of Social Sciences/Health Sciences, University of Tampere, Tampere, Finland

[10]Center for Child Health Research, University of Tampere, Tampere University Hospital, Tampere, Finland

[11]The Science Center, Pirkanmaa Hospital District, Tampere, Finland

[12]Department of Pediatrics, PEDEGO Research Unit, Medical Research Center, Oulu University Hospital and University of Oulu, Oulu, Finland

[13]Immunogenetics Laboratory, Institute of Biomedicine, University of Turku, Turku, Finland

[14]Clinical Microbiology, Turku University Hospital, Turku, Finland

[15]Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

[16]Research Programs Unit, Diabetes and Obesity, University of Helsinki, Helsinki, Finland

[17]Folkhälsan Research Center, Helsinki, Finland

[18]Department of Pediatrics, Tampere University Hospital, Tampere, Finland

Correspondence
Laura Korhonen, Department of Dermatology, Tampere University Hospital, Tampere, Finland.
Email: laura.korhonen@sll.fimnet.fi

Funding information
This work was funded by the European Comission Persistent Virus Infection in Diabetes Network [PEVNET] Frame Programme 7Contract No. 261441, Tampere Tuberculosis Foundation, Sigrid Juselius Foundation, Academy of Finland [PREVALLER consortium, Grant 129448-2008; Research Grant 132362-2009; Centre of Excellence in Molecular Systems Immunology and Physiology, Grant 250114 2012–2017], The National Technology Agency in Finland (TEKES), Päivikki and

Summary

Background: Prenatal environment has been shown to influence child's risk of atopic diseases. Laboratory-confirmed data about the role of maternal infections during pregnancy is scarce.

Objective: The aim of this study was to determine the associations between serologically confirmed maternal infections during pregnancy and atopic disease in the offspring.

Methods: This was a nested case-control study within a prospective birth cohort study. Altogether 202 atopic case children and 333 matched non-atopic control children were included. Atopic outcome was defined as having an atopic disease and IgE sensitization by the age of 5 years. We analysed serologically acute enterovirus (EV), influenza virus A (IAV) and *Mycoplasma pneumoniae* (*M. pneumoniae*) infections

Sakari Sohlberg Foundation, The Finnish Medical Foundation and Finnish Cultural Foundation.

during pregnancy, and mother's seropositivity against human cytomegalovirus (CMV) and *Helicobacter pylori*.

Results: Maternal EV infection during pregnancy was inversely associated with atopic outcome in the offspring (odds ratio 0.43; 95% confidence interval: 0.23-0.80, $P = 0.008$). Acute IAV or *M. pneumoniae* infections or seropositivity against CMV or *Helicobacter pylori* were not associated with the atopic outcome.

Conclusions and Clinical Relevance: Our results suggest that maternal EV infections during pregnancy are inversely associated with atopic disease in the offspring. Our finding provides further support to the previous studies suggesting an important role of the *in utero* environment in the development of atopic diseases.

1 | INTRODUCTION

Genetic factors regulate the susceptibility to atopic diseases but the environment has a significant impact on the disease development. Atopic diseases usually manifest in childhood stressing the importance of environmental factors present in very early life.[1] Accordingly, extensive data suggest that a variety of environmental exposures in early childhood can influence the development of atopic diseases.[2] Postnatal microbial infections have been among the most studied factors and several reports have suggested a modulation of the risk of atopic diseases by childhood infections.[3-11]

Recently, it has become increasingly evident that already the *in utero* environment may be important in the development of atopic diseases.[2] A number of prenatal factors, for example, maternal smoking,[12] adverse life events[13] and pre-pregnancy overweight[14] have been associated with increased risk of atopic diseases in the offspring. In contrast, maternal exposure to farming environment has been shown to protect the child from atopy.[15-17] These findings, consistent with the framework of hygiene hypothesis, raise the question about the role of prenatal microbial infections in atopy.

The majority of previous data on prenatal infections are derived from questionnaire-based studies, most of which report an increased risk of atopic disease in children born to mothers with febrile or flu-like infections during pregnancy.[18] However, questionnaire-based studies do not enable the identification of culprit microbes and they may be affected by recall bias. Applying laboratory assay-based methods can overcome these shortcomings but such studies are scarce.[19-22] Maternal gastrointestinal helminth infections have been reported both to inversely associate with eczema in the offspring[20] and not to associate with atopy.[19] Intrauterine bacterial growth at birth as well as chorioamnionitis has been shown to increase the risk of asthma in the offspring.[21,23]

We wanted to investigate whether laboratory-confirmed prenatal infections are associated with atopic outcome in the offspring. In addition, we aimed to determine whether microbes with different infectious and immunological reaction patterns differ in their effect. Five microbes, featuring gastrointestinal and respiratory pathogens, as well as microbes causing acute and chronic infections were included in the current study.

Enteroviruses (EV) can replicate in the gastrointestinal tract, and they have been linked to atopy in some studies[6-8] but not all.[4,5] *Helicobacter pylori* (*H. pylori*), causing chronic gastric infections and inflammation, and also considered to be a marker of the general hygiene level, has been shown to associate with atopy.[24] Influenza virus A (IAV) causes a strong acute systemic infection, whereas cytomegalovirus (CMV) persists as a lifelong latent infection. *Mycoplasma pneumoniae* (*M. pneumoniae*) in turn causes often subclinical, chronic lower respiratory tract infections. We analysed acute gestational EV, IAV and *M. pneumoniae* infections by serology from paired serum samples taken during pregnancy. We estimated the incidence of acute *H. pylori* and CMV infections during pregnancy to be very low in the Finnish population and therefore chose to analyse the presence of these chronic/latent infections from a single serum sample.

2 | MATERIALS AND METHODS

2.1 | Subjects

The study cohort was derived from the prospective Type 1 Diabetes Prediction and Prevention (DIPP) study in Finland.[25] In the DIPP study, newborn infants with HLA conferred susceptibility to type 1 diabetes are invited to enter a prospective follow-up with visits at the study clinic every 3-12 months. At each visit, children undergo a comprehensive interview and clinical examination. Biological samples, including venous blood samples, are collected according to the study protocol.

In the present study, we first searched the DIPP database for children fulfilling the atopic outcome criteria applied: Diagnosis of bronchial asthma, atopic dermatitis and/or allergic rhinitis and a positive serum IgE level against aeroallergens at the age of 5 years. Altogether, 202 atopic case children were identified. They were born between June 1996 and September 2004 in the regions of Tampere and Oulu cities. Next, we selected 1-2 non-atopic control children for each case child having neither specific IgE against aeroallergens nor diagnosis of any atopic disease (n = 333). Case and control children were matched for gender, region of birth, time of birth (± 3 months) and type 1 diabetes-related HLA-DQB1 alleles. In total,

128 (63%) case children and 207 (62%) control children were boys. The mean age difference between cases and controls was 45 days (SD 34 days, range 0-91 days), and the percentages of case and control children born each season of the year were equal. Altogether, 9% of children carried the HLA-DQB1 *02/*03:02 genotype and 91% had the *03:02/x genotype with x referring to other alleles than *02, *03:01 or *06:02.

Paired serum samples were available from each mother of participating children. First sample was taken in prenatal clinics at the end of the first trimester of pregnancy as a part of a national screening for infectious diseases. These samples are stored in the nationwide Finnish Maternity Cohort biobank. The second sample was cord blood serum from the newborn infant collected according to DIPP study protocol. The Finnish Maternity Cohort Steering Group at the National Institute for Health and Welfare approved the use of biobank samples in this study. The DIPP study protocol has been approved by the ethical committees of the participating university hospitals (ETL 97193M), and parents have given written informed consent.

2.2 | IgE antibodies

IgE antibodies against a mixture of common aeroallergens were analysed from the serum samples taken at the age of 5 years with ImmunoCAP® enzyme immunoassay (Phadia AB, Uppsala, Sweden). The multi-allergen test used (Phadiatop®, Phadia AB, Uppsala, Sweden) contains allergens of common pollens, moulds and animals. Values of ≥0.35 kU/L were considered positive.

2.3 | Microbial analyses

IgG class antibodies against EV, IAV, *H. pylori* and *M. pneumoniae* were measured by applying enzyme immune assays (EIA). IgG class antibodies against CMV were measured either by applying EIA or by chemiluminescent immunoassay (CLIA). For CMV, two different assays were used due to changes in laboratory equipment during the study period but each case-control pair was always analysed with the same method.

Enterovirus, IAV and *M. pneumoniae* antibodies were analysed from both first trimester serum samples and cord blood, *H. pylori* antibodies from cord blood and CMV antibodies from first trimester serum samples (n = 146) or cord blood (n = 381), whichever was available. Samples from case-control pairs and related first trimester and cord blood samples were analysed in parallel in the same test run. All analyses were carried out blind to clinical information.

The assay for EV antibodies employed a synthetic EV peptide that carries an immunodominant epitope of the viral VP1 protein (sequence KEVPALTAVETGAT-C) as an antigen, as described.[26] This epitope is highly conserved among EVs detecting antibodies against a wide range of different EV types. For IAV, we used influenza A virus strain Beijing (BA1231VS, Virion Serion, Würzburg, Germany) as an antigen, as previously described.[27] In brief, microtiter plates (Nunc Immuno™ plate, Maxisorb, Thermo Fisher Scientific, Waltham, MA, USA) were coated by the antigens at concentrations 1 μg/mL for EV and 3 μg/mL for IAV in carbonate buffer (pH 9.4). Serum samples were analysed diluted 1:1000 in PBS supplemented with 1% bovine serum albumin, 2% NaCl and 0.05% Tween 20. Serial dilutions for strong positive serum samples were used to reach standard curve range. Binding of antibodies was documented by using peroxidase-conjugated anti-human IgG (P214, Dako, Glostrup, Denmark) and measuring the absorbance at 490 nm. The results were given in enzyme immunoassay units (EIU) with reference to negative and positive control samples. An EIU value of 15 was applied as a cut-off level for seropositivity as described.[26]

Antibodies against *H. pylori* (Enzygnost® Anti-Helicobacter pylori/IgG, Siemens, Marburg, Germany) and antibodies against *M. pneumoniae* (Mycoplasma pneumonia IgG, Labsystems Diagnostics Ltd, Helsinki, Finland) were measured using commercial kits according to the manufacturers' instructions. For the analysis of antibodies against CMV from the first trimester samples, we applied Enzygnost® Anti-CMV/IgG EIA kit (Siemens), and for cord blood samples, we used LIAISON® CMV IgG II CLIA (DiaSorin S.p.A., Saluggia (VC), Italy) according to the manufacturers' instructions. We used Siemens BEP III (Siemens) for processing and calculation of the antibody levels for the EIAs and LIAISON® XL (DiaSorin S.p.A.) for CLIA.

2.4 | Definition of acute infection

According to the manufacturer's instructions, we defined an acute *M. pneumoniae* infection as a 1.6-fold or higher increase in antibody level between the maternal first trimester sample and the child's cord blood sample. For cohesion, we applied the same criteria also for EV and IAV to indicate an acute infection during pregnancy.

2.5 | Statistical methods

We applied conditional logistic regression analysis to determine the association between an acute infection during pregnancy (EV, IAV, *M. pneumoniae*) or seropositivity (CMV, *H. pylori*) and atopic disease. The results are presented as odds ratios (OR) and 95% confidence intervals (CI) for atopic disease.

Demographic factors are presented in Table 1. We applied conditional regression analysis to estimate the individual association of each variable with atopic outcome. When an association was observed, conditional regression analysis was applied to adjust for these factors. If a value was missing from the case child or all controls in the case-control group, that case-control pair/triplet was excluded from the analyses.

We applied the Bonferroni correction to counteract the problem of multiple comparisons. As we analysed the association between acute infection during pregnancy and atopic disease for three microbes, the Bonferroni correction to control type I error was justified. After applying the Bonferroni correction, that is, dividing $P = 0.05$ by 3, P-values < 0.017 were regarded as statistically significant. Similarly, as seropositivity in a single serum sample was analysed for two microbes, after applying Bonferroni correction, P-

TABLE 1 Frequencies of potential confounders in case and control children and associations with the study outcome

	Case n = 202 (%)	Control n = 333 (%)	OR (95% CI)	*P* value
Older siblings (yes)	95 (47)	201 (60)	0.60 (0.42-0.86)	**0.006**
Furry pets (yes)	58 (29)	146 (44)	0.53 (0.36-0.78)	**0.001**
Smoking in pregnancy (yes)	17 (9)	28 (8)	1.00 (0.53-1.89)	1.00
Maternal education				
No secondary	50 (27)	98 (31)	ref	
Lower secondary	91 (48)	151 (48)	1.01 (0.65-1.59)	0.96
Higher secondary	48 (25)	63 (20)	1.26 (0.76-2.11)	0.37
Paternal education				
No secondary	86 (46)	144 (48)	ref	
Lower secondary	46 (25)	88 (30)	0.92 (0.59-1.44)	0.71
Higher secondary	56 (30)	66 (22)	1.34 (0.85-2.11)	0.21
Duration of pregnancy[ab]	280 (224-300)	280 (209-300)	1.01 (0.99-1.02)	0.53
Birth weight[ac]	3650 (1910-4830)	3610 (1660-5540)	1.00 (1.00-1.00)	0.27

OR and 95% CI were estimated using conditional logistic analysis. *P* values below 0.05 are marked in bold.
[a]Values are medians (minimum and maximum).
[b]Presented in days.
[c]Presented in grams.

values < 0.025 were statistically significant. Unadjusted *P*-values are presented in the text.

Analyses were performed by using R version 3.3.3 (2017-03-06, The R Foundation for Statistical Computing, https://www.R-project.org).

3 | RESULTS

Out of the 202 case children, 52 (26%) had asthma, 126 (62%) had atopic eczema and/or allergic rhinitis, and 24 (12%) had both asthma and eczema/rhinitis. Specific IgE values varied between 0.36 and 101 kU/L (mean 22.9, median 11). Demographics and their associations with atopic outcome are presented in Table 1.

A gestational EV infection was observed in 17 (8%) mothers of the case children and in 53 (16%) mothers of the control children (Figure 1). *M. pneumoniae* infection was detected in 14 (7%) vs 37 (11%), and IAV in 26 (13%) vs 43 (13%) mothers of case and control children, respectively. Seropositivity against CMV was observed in 148 (74%) vs 243 (74%), and *H. pylori* in 14 (7%) vs 37 (11%) mothers of the case and control children, respectively.

Maternal EV infection during pregnancy was inversely associated with atopic outcome in the offspring (OR: 0.43; 95% CI: 0.23-0.80; *P* = 0.008). The result remained statistically significant after adjusting for the relevant confounding factors, that is, older siblings and furry pets (OR: 0.43; 95% CI: 0.23-0.81; *P* = 0.009) (Figure 2). Maternal IAV or *M. pneumoniae* infections during pregnancy did not associate with atopic outcome (OR: 1.05; 95% CI: 0.62-1.79 and OR: 0.65; 95% CI: 0.34-1.22, respectively). When all the three microbes were analysed together, 49 (24%) case mothers and 115 (35%) control mothers had experienced at least one acute infection during pregnancy (OR: 0.60; 95% CI: 0.39-0.90; *P* = 0.015).

Maternal seropositivity against CMV or *H. pylori* was not associated with atopic outcome in the offspring (OR: 0.99; 95% CI: 0.66-1.47 and OR: 0.58; 95% CI: 0.30-1.12, respectively).

4 | DISCUSSION

The current study suggests that maternal EV infections during pregnancy are inversely associated with atopic disease in the offspring. To our knowledge, EVs or other microbes included have not been studied in a similar laboratory-based study setting previously.

We did not find any association between prenatal IAV and *M. pneumoniae* infections and atopic outcome. One reason may lie in the route of infection; IAV and *M. pneumoniae* are respiratory microbes, whereas EVs can replicate also in the gut. There are indications that exposures to enteric pathogens might be particularly important in the development of atopy and they have been reported to inversely associate with atopy in some studies[28,29] but not in all.[3,11] Our group has previously observed an inverse association between neutralizing antibodies against echoviruses, belonging to the enterovirus genus, and atopy.[6,7] A recent study showed reduced anti-echovirus 30 antibody titres in asthmatic children as compared with non-asthmatics,[8] which was contrary to a previous rhinovirus antibody finding.[9] In addition, some EVs replicate for relatively long periods in the gut-associated lymphoid tissue that is believed to be important in maintaining immunological tolerance, and EV infections have been shown to associate with tolerogenic immune responses, for example, production of IL-10.[30]

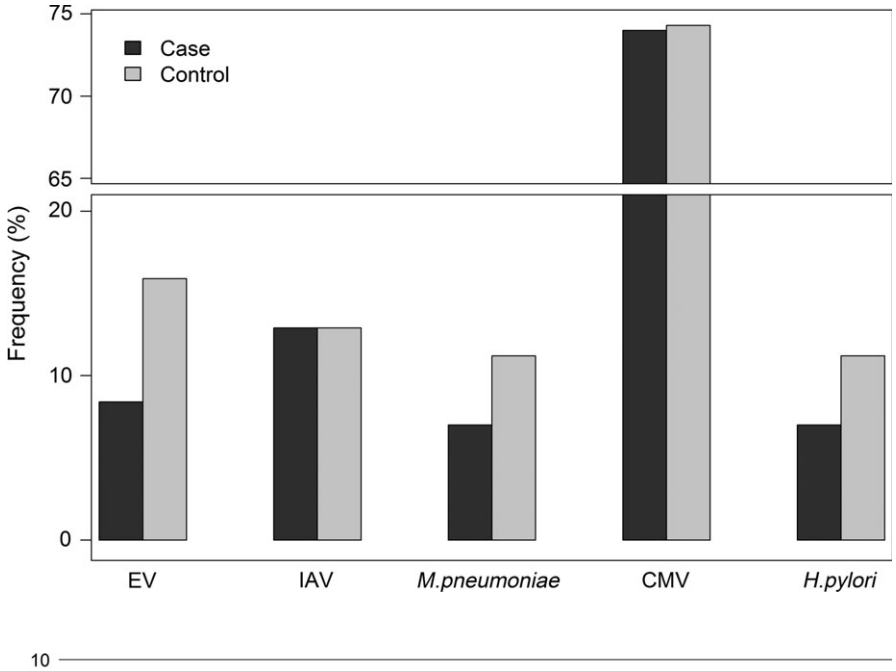

FIGURE 1 The proportion (%) of case and control mothers with an acute EV, IAV or *M. pneumoniae* infection during pregnancy or seropositivity against CMV or *H. pylori*. CMV, cytomegalovirus; EV, enterovirus; *H. pylori*, *Helicobacter pylori*; IAV, influenza A virus; *M. pneumoniae*, *Mycoplasma pneumoniae*

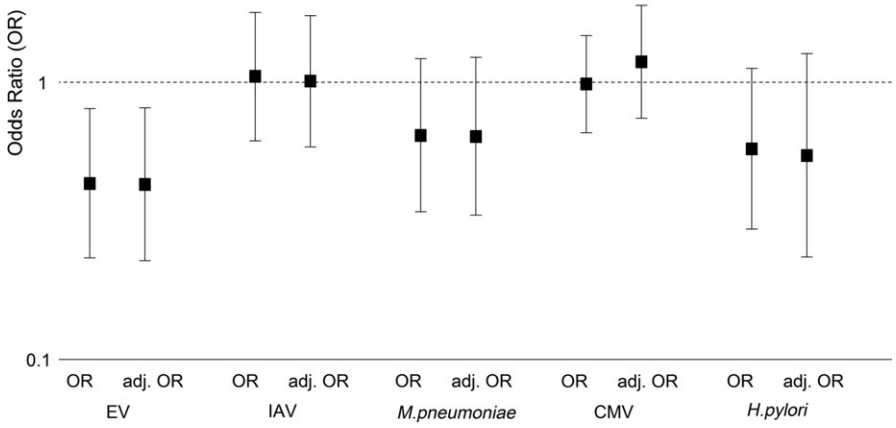

FIGURE 2 The associations between acute infections during pregnancy (EV, IAV and *M. pneumoniae*) or mother's seropositivity (CMV and *H. pylori*) and atopic disease in the offspring. Results are presented as OR and 95% CI for each microbe before and after adjusting for relevant confounding factors, that is, having older siblings and contact with furry animals (adj. OR). CMV, cytomegalovirus; EV, enterovirus; *H. pylori*, *Helicobacter pylori*; IAV, influenza A virus; *M. pneumoniae*, *Mycoplasma pneumoniae*

Maternal microbial infections during pregnancy can affect the fetus in several ways. Viral infections can cross the placental barrier or infect the placenta, which can have severe consequences for the fetus.[31-33] Infections can also affect the fetus through a local inflammatory response in the placenta or activation of the maternal systemic immune response.[31] For instance, toll-like receptors (TLRs) are an important part of innate immunity against viruses and the type of TLR activated is suggested to depend on the mechanism of replication used by the virus.[34] Maternal exposure to farming has been shown to increase gene expression of certain TLRs in cord blood,[17] and increased expression of some TLR genes at birth has been associated with a reduced risk of atopy in the offspring.[16] There are also some indications that maternal infections during pregnancy might affect the placental microbiome,[35] that may in turn influence the microbial composition of the newborn infant's first intestinal discharge (meconium).[36] Differential activation of innate immunity or changes in the composition of early microbiota might be possible mechanisms behind the findings in our study and offer an interesting field for future research.

The strengths of this study include the prospective study setting, availability of paired serum samples taken during pregnancy and the strict definition of atopic outcome including both IgE sensitization and a clinically relevant atopic disease. Most importantly, we relied on serologically confirmed infections. Many infections, including EV infections, are asymptomatic or cause only mild clinical presentations, but serological assays are able to capture both symptomatic and silent infections. There are only a few previous studies with laboratory-confirmed infections during pregnancy, and to our

knowledge, the microbes included in the present study have not been studied previously in detail.[19-21] In a study by Murphy *et al*,[22] they used PCR to detect viruses (including EVs and IAVs) from nasal swabs taken from pregnant asthmatic mothers during a symptomatic upper respiratory infection. They reported that infants born to mothers with nasal swab positive for any of the tested viruses had an increased risk of atopy as compared to virus negative mothers. However, numbers of detected viruses were relatively small; for example, only 3 EVs detected in 42 samples, and therefore, no conclusions could be drawn about the role of individual viruses. In the present study, we used systematic serological screening to detect both symptomatic and asymptomatic infections as well as infections that did not coincide with the sample draws.

Acute gestational infections were diagnosed by increases in IgG levels between maternal serum samples taken at the end of the first trimester and cord blood serum. The time interval between the two samples was longer than that usually applied in clinical diagnostics, but it enabled us to capture acute gestational infections as extensively as possible. IgG responses last usually for several months or years, but it is possible that some infections may have remained undetectable due to low or short IgG responses. However, this should have occurred similarly in both case and control groups. We used cord blood serum as the second sample, since no maternal serum was taken at delivery. We have previously shown that EV IgG levels in cord blood sera correlate well with IgG levels in maternal sera at the time of delivery.[37] We did not measure IgM antibodies, since IgM antibodies do not cross the placenta and IgM responses do not develop in all EV infections leading to diminished sensitivity.[38]

There are some limitations of our study. First, lack of information on parental history of atopy prevented us from analysing the results with respect to different genetic backgrounds. Therefore, the possibility that atopic mothers are genetically less susceptible to EV infections or that there is another immunological or environmental factor affected by maternal atopy status, cannot be excluded. Secondly, our study population was selected for type 1 diabetes-associated HLA genotypes, which could affect the generalizability of the results. Although HLA-DQ region has been linked to asthma, the genetic backgrounds of IgE sensitization and atopic diseases are highly polymorphic with no strong association to HLA-DQ region.[39] Therefore, we find it unlikely that the HLA selection would substantially influence our results. Furthermore, the case and control groups were matched for the type 1 diabetes-associated HLA genotypes. It should also be noted that the prevalence of acute EV infections during pregnancy was found to be relatively low, being in line with previous studies.[37,40] As atopic diseases are common in childhood, it is likely that gestational EV infections are not a major risk-modifying factor in atopy. However, the observational design of the present study makes it difficult to estimate the potential size of this effect on population level reliably. One should also note that even though the specificity of the current EV antibody assay has been well documented,[41] we cannot completely exclude the possibility that in some cases, it could have detected antibodies against other viruses than EVs. Finally, we have not addressed the mechanisms behind the inverse association between gestational EV infections and atopy, and therefore, it is possible that EVs are merely a surrogate marker for a certain kind of environment mediating the effect observed.

In conclusion, our study suggests that maternal EV infections during pregnancy are inversely associated with atopic disease in the offspring. Other included microbes causing acute or chronic infections lacked this association suggesting that microbes differ in their capability to affect the processes involved in the development of atopy. This stresses the importance of microbe-specific identification of infections when evaluating their role in the pathogenesis of atopy. Previous research has shown that postnatal infections play a role in the development of atopy, and our study adds to this knowledge by suggesting that microbial exposure already *in utero* might also be of importance. Thus, addressing the overall effect of gestational and postnatal infections on the development of atopy is an important objective for future studies.

ACKNOWLEDGEMENTS

We thank the DIPP study group and study nurses. We are deeply grateful to Prof. Olli Simell for his valuable contribution to the DIPP study and to Prof. Jorma Toppari as the current principal investigator of the Turku clinical DIPP centre. We are thankful to Eveliina Jalonen, Aaro Piirainen, Tuula Ruuti, Irma Valtonen and Sini Wallenius for their skilful technical assistance. We also thank the local study personnel and the participating families for their collaboration.

CONFLICT OF INTEREST

Professors Hyöty and Knip are minor shareholders of Vactech Ltd developing vaccines against picornaviruses. Other authors declare no conflict of interest.

ORCID

Laura Korhonen http://orcid.org/0000-0002-5566-8054

REFERENCES

1. Holt PG. The mechanism or mechanisms driving atopic asthma initiation: the infant respiratory microbiome moves to center stage. *J Allergy Clin Immunol*. 2015;136:15-22.
2. Lodge CJ, Dharmage SC. Breastfeeding and perinatal exposure, and the risk of asthma and allergies. *Curr Opin Allergy Clin Immunol*. 2016;16:231-236.
3. Bodner C, Anderson WJ, Reid TS, Godden DJ. Childhood exposure to infection and risk of adult onset wheeze and atopy. *Thorax*. 2000;55:383-387.
4. Kotaniemi-Syrjanen A, Vainionpaa R, Reijonen TM, Waris M, Korhonen K, Korppi M. Rhinovirus-induced wheezing in infancy-the first sign of childhood asthma? *J Allergy Clin Immunol*. 2003;111:66-71.
5. Jackson DJ, Evans MD, Gangnon RE, et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *Am J Respir Crit Care Med*. 2012;185:281-285.

6. Seiskari T, Kondrashova A, Tauriainen S, et al. Role of enterovirus infections in IgE sensitization. *J Med Virol*. 2012;84:268-271.
7. Korhonen L, Kondrashova A, Tauriainen S, et al. Enterovirus infections in early childhood and the risk of atopic disease-a nested case-control study. *Clin Exp Allergy*. 2013;43:625-632.
8. Iwasaki J, Chai LY, Khoo S, et al. Lower anti-echovirus antibody responses in children presenting to hospital with asthma exacerbations. *Clin Exp Allergy*. 2015;45:1523-1530.
9. Iwasaki J, Smith W, Khoo S, et al. Comparison of rhinovirus antibody titers in children with asthma exacerbations and species-specific rhinovirus infection. *J Allergy Clin Immunol*. 2014;134:25-32.
10. Lee ZM, Huang YH, Ho SC, Kuo HC. Correlation of symptomatic enterovirus infection and later risk of allergic diseases via a population-based cohort study. *Medicine (Baltimore)*. 2017;96:e5827.
11. Reimerink J, Stelma F, Rockx B, et al. Early-life rotavirus and norovirus infections in relation to development of atopic manifestation in infants. *Clin Exp Allergy*. 2009;39:254-260.
12. Silvestri M, Franchi S, Pistorio A, Petecchia L, Rusconi F. Smoke exposure, wheezing, and asthma development: a systematic review and meta-analysis in unselected birth cohorts. *Pediatr Pulmonol*. 2015;50:353-362.
13. Hartwig IRV, Sly PD, Schmidt LA, et al. Prenatal adverse life events increase the risk for atopic diseases in children, which is enhanced in the absence of a maternal atopic predisposition. *J Allergy Clin Immunol*. 2014;134:160-169.
14. Zugna D, Galassi C, Annesi-Maesano I, et al. Maternal complications in pregnancy and wheezing in early childhood: a pooled analysis of 14 birth cohorts. *Int J Epidemiol*. 2015;44:199-208.
15. Ege MJ, Bieli C, Frei R, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *J Allergy Clin Immunol*. 2006;117:817-823.
16. Roduit C, Wohlgensinger J, Frei R, et al. Prenatal animal contact and gene expression of innate immunity receptors at birth are associated with atopic dermatitis. *J Allergy Clin Immunol*. 2011;127:179-185.
17. Loss G, Bitter S, Wohlgensinger J, et al. Prenatal and early-life exposures alter expression of innate immunity genes: the PASTURE cohort study. *J Allergy Clin Immunol*. 2012;130:523-530.
18. Zhu T, Zhang L, Qu Y, Mu D. Meta-analysis of antenatal infection and risk of asthma and eczema. *Medicine (Baltimore)*. 2016;95:e4671.
19. Cooper PJ, Chico ME, Amorim LD, et al. Effects of maternal geohelminth infections on allergy in early childhood. *J Allergy Clin Immunol*. 2016;137:899-906.
20. Mpairwe H, Ndibazza J, Webb EL, et al. Maternal hookworm modifies risk factors for childhood eczema: results from a birth cohort in Uganda. *Pediatr Allergy Immunol*. 2014;25:481-488.
21. Keski-Nisula L, Katila M, Remes S, Heinonen S, Pekkanen J. Intrauterine bacterial growth at birth and risk of asthma and allergic sensitization among offspring at the age of 15 to 17 years. *J Allergy Clin Immunol*. 2009;123:1305-1311.
22. Murphy VE, Mattes J, Powell H, Baines KJ, Gibson PG. Respiratory viral infections in pregnant women with asthma are associated with wheezing in the first 12 months of life. *Pediatr Allergy Immunol*. 2014;25:151-158.
23. Kumar R, Yu Y, Story RE, et al. Prematurity, chorioamnionitis, and the development of recurrent wheezing: a prospective birth cohort study. *J Allergy Clin Immunol*. 2008;121:878-884.
24. Taye B, Enquselassie F, Tsegaye A, Medhin G, Davey G, Venn A. Is *Helicobacter Pylori* infection inversely associated with atopy? A systematic review and meta-analysis. *Clin Exp Allergy*. 2015;45:882-890.
25. Näntö-Salonen K, Kupila A, Simell S, et al. Nasal insulin to prevent type 1 diabetes in children with HLA genotypes and autoantibodies conferring increased risk of disease: a double-blind, randomised controlled trial. *Lancet*. 2008;372:1746-1755.
26. Hyöty H, Hiltunen M, Knip M, et al. A prospective study of the role of Coxsackie B and other enterovirus infections in the pathogenesis of IDDM. *Diabetes*. 1995;44:652-657.
27. Kondrashova A, Nurminen N, Patrikainen M, et al. Influenza A virus antibodies show no association with pancreatic islet autoantibodies in children genetically predisposed to type 1 diabetes. *Diabetologia*. 2015;58:2592-2595.
28. Linneberg A, Ostergaard C, Tvede M, et al. IgG antibodies against microorganisms and atopic disease in Danish adults: the Copenhagen Allergy Study. *J Allergy Clin Immunol*. 2003;111:847-853.
29. Matricardi PM, Rosmini F, Riondino S, et al. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ*. 2000;320:412-417.
30. Abdel-Latif M, Abdel-Moneim AA, El-Hefnawy MH, Khalil RG. Comparative and correlative assessments of cytokine, complement and antibody patterns in paediatric type 1 diabetes. *Clin Exp Immunol*. 2017;190:110-121.
31. Racicot K, Mor G. Risks associated with viral infections during pregnancy. *J Clin Invest*. 2017;127:1591-1599.
32. Ornoy A, Tenenbaum A. Pregnancy outcome following infections by coxsackie, echo, measles, mumps, hepatitis, polio and encephalitis viruses. *Reprod Toxicol*. 2006;21:446-457.
33. Satosar A, Ramirez NC, Bartholomew D, Davis J, Nuovo GJ. Histologic correlates of viral and bacterial infection of the placenta associated with severe morbidity and mortality in the newborn. *Hum Pathol*. 2004;35:536-545.
34. Carty M, Bowie AG. Recent insights into the role of Toll-like receptors in viral infection. *Clin Exp Immunol*. 2010;161:397-406.
35. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. *Sci Transl Med*. 2014;6:237ra65.
36. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Sci Rep*. 2016;6:23129.
37. Sadeharju K, Knip M, Virtanen SM, et al. Maternal antibodies in breast milk protect the child from enterovirus infections. *Pediatrics*. 2007;119:941-946.
38. Harvala H, Broberg E, Benschop K, et al. Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. *J Clin Virol*. 2018;101:11-17.
39. Moffatt MF, Schou C, Faux JA, et al. Association between quantitative traits underlying asthma and the HLA-DRB1 locus in a family-based population sample. *Eur J Hum Genet*. 2001;9:341-346.
40. Viskari H, Knip M, Tauriainen S, et al. Maternal enterovirus infection as a risk factor for type 1 diabetes in the exposed offspring. *Diabetes Care*. 2012;35:1328-1332.
41. Samuelson A, Glimaker M, Skoog E, Cello J, Forsgren M. Diagnosis of enteroviral meningitis with IgG-EIA using heat-treated virions and synthetic peptides as antigens. *J Med Virol*. 1993;40:271-277.

How to cite this article: Korhonen L, Seiskari T, Lehtonen J, et al. Enterovirus infection during pregnancy is inversely associated with atopic disease in the offspring. *Clin Exp Allergy*. 2018;48:1698–1704. https://doi.org/10.1111/cea.13280